Protection of Mice Against a Lethal Challenge by Influenza Virus: Development of Influenza Vaccines Intended for Human Use

References and Abstracts

1: Infect Immun 1976 Mar;13(3):818-24

Aerosol vaccination of mice with a live, temperature- sensitive recombinant influenza virus.

Jemski JV, Walker JS.

Mice were vaccinated intranasally (i.n.) or with small-particle aerosols (SPA; 2 mum) or large-particle aerosols (LPA; 8 mum) of an attenuated, temperature-sensitive, recombinant A influenza (H3N2) virus, ts-1 (E). Serum virus-neutralizing and hemagglutination inhibition antibodies were detected for all vaccinated mice by 28 days. Bronchoalveolar wash fluids had increased levels of immunoglobulin (IgG, IgA) only in the i.n. -vaccinated mice. Hemagglutination and virus-neutralizing antibodies were detected in the SPA- and i.n. -vaccinated groups but not in the LPA vaccinates. Upon challenge with SPA of a mouse virulent H3N2 influenza vitus, total protection was obtained for the SPA- and I.N. -vaccinated mice, whereas only 89% of the LPA group survived. Replication of the challenge virus was signifcantly repressed in both the lower and upper respiratory tracts of the three groups of vaccinated mice compared to the nonvaccinated controls. The protection afforded the SPA- and i.n. -vaccinated mice was the same as measured for mice after recovery from earlier subelthal active infection with virulent virus.

2: Dev Biol Stand 1975;28:336-9

Potency of influenza vaccines: mouse protection experiments in correlation to field studies in man.

Bachmayer H, Liehl E, Schmidt G.

The seroconversion rates have been studied following vaccination of human volunteers with two commercial influenza vaccines. Vaccine A did not give a significant increase of hemagglutination-inhibition titers. Vaccine B, on the other hand, raised the titers 2- to 8- fold, depending on the pretiters of the individuals. The potency of the same vaccines has been tested using mouse protection experiments: vaccine B gave significantly better protection rates, as measured by survival as well as by reduction of lung lesions. These results give additional evidence that the use of mouse protection experiments for the evaluation of different influenza vaccines is meaningful.

3: Dev Biol Stand 1975;28:319-23

Effect of neuraminidase on potency of inactivated influenza virus vaccines in mice.

Reichert E, Mauler R.

The protective effect of neuraminidase was studied in a mouse protection test using isolated neuraminidase of A2/Aichi/68(H3N2) virus and the complete recombinant virus A/eq(Heq-1)-HK(N2) as antigens. Immunized mice were protected against A2/Aichi(H3N2) challenge virus; however, the protection rate was low in comparison to animals immunized with comparable amounts of the complete A2/68(H3N2) virus9 Furthermore there was no cross-protection against A2/Asia/57(H2N2) challenge virus. The protective effect of neuraminidase was not impaired by Tween-ether treatment of the A/eq(Heq-1)-HK(N2) recombinant virus.

4: J Infect Dis 1982 Mar;145(3):320-30

Evaluation of live and inactivated influenza A virus vaccines in a mouse model.

Armerding D, Rossiter H, Ghazzouli I, Liehl E.

Induction of cross-protective immunity against serologically distinct subtypes of influenza A virus in mice was examined in an attempt to correlate cross-protection with heterotypic lymphocyte responses. Live and inactivated virus vaccines protected against the homologous subtype, but only whole virus protected against heterologous subtypes. Live virus vaccines provided better cross-protection than inactivated virus vaccines. A weak defense against heterotypic challenge generated by live H0N1 virus could be boosted by cross-stimulation with whole H3N2 virus and by restimulation with pathogenic H0N1 virus. Heterotypic protection persisted for at least five months. Live viruses induced cross-reactive cytotoxic T cells in normal mice. However, cross-stimulation with heterologous virus was required to generate secondary cytotoxicity. Cross-reactive B lymphocytes were evident after inoculation with whole virus.

5: Arch Virol 1981;70(2):83-9

Comparative immunogenicity of live influenza viruses and their solubilized neuraminidases: results of mouse protection experiments.

Bottex C, Burckhart MF, Fontanges R.

Antigenicity and immunogenicity of three influenza virus strains A/PR/8/34 (H1N1). A/Hong Kong/1/68 (H3N2) and A/Port Chalmers/1/73 (H-3H2) were assayed comparatively with their corresponding neuraminidase isolated by proteolysis, and with the recombinant virus X-42 (Heq1 N2). It was concluded that intranasal immunization of mice with live virus induced heterologous immunity. Except in homologous neuraminidase-vaccinated mice, the subunit always was shown less effective and demonstrated a significantly lower antibody response than the corresponding whole virus.

6: J Virol 1995 Dec;69(12):7712-7

Antibody-forming cell response to virus challenge in mice immunized with DNA encoding the influenza virus hemagglutinin.

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Immunization of mice with DNA encoding the influenza virus hemagglutinin (HA) affords complete protection against lethal influenza virus infection and the means to investigate the mechanisms of B-cell responsiveness to virus challenge. Using a single-cell enzyme-linked immunospot assay, we sought to determine the localization of HA-specific antibody-forming cells (AFCs) during the development of humoral immunity in mice given HA DNA vaccine by gene gun. At 33 days postvaccination, populations of AFCs were maintained in the spleen and bone marrow. In response to lethal challenge with influenza virus, the AFCs became localized at the site of antigenic challenge, i.e., within the draining lymph nodes of the lung compartment. Immunoglobulin G (IgG)- and IgA-producing AFCs were detected in lymph nodes of the upper and lower respiratory tracts, underscoring their importance in clearing virus from the lungs. Response to challenge required competent CD4+ T cells, without which no AFCs were generated, even those producing IgM. By contrast, in mice vaccinated with an HA-containing subunit vaccine, fewer AFCs were generated in response to challenge, and these animals were less capable of resisting infection. Our findings demonstrate the comparable localization of AFCs in response to challenge in mice vaccinated with either HA DNA or live virus. Moreover, the former strategy generates both IgG- and IgA-producing plasma cells.

7: J Infect Dis 1977 Dec;136 Suppl:S712-7

Evaluation of commercially prepared vaccines for experimentally induced type A/New Jersey/8/76 influenza virus infections in mice and squirrel monkeys.

Berendt RF, Scott GH.

Mice and squirrel monkeys were vaccinated and subsequently challenged at selected times to evaluate the immunoprophylactic value of vaccines against influenza virus type A/New Jersey/76. Mice were challenged with virulent, homologous virus either 17 or 60 days after vaccination with 80 chick cell-agglutinating (CCA) units of whole-virus vaccine. Vaccinated mice showed minimal lesions and virus in lung tissue and had lower lung weights than unvaccinated controls. These mice had titers of hemagglutination-inhibiting (HAI) antibody in serum of greater than 1:400, but only traces of antibody were found in lung washes. Vaccinated squirrel monkeys had significantly less illness than unvaccinated controls when challenged with virulent virus 30 days after intramuscular immunization with 200 CCA units of whole virus or 400 CCA units of split virus given either once or twice (at 30-day intervals). Equal protection was observed in all monkeys despite the absence of serum HAI antibody in some monkeys after vaccination. Anamnestic reactions were observed only in monkeys vaccinated with whole virus. The possible roles of various immune factors and antibody to neuraminidase are discussed.

8: J Clin Immunol 1987 Jul;7(4):327-32

Age-dependent antibody response in mice and humans following oral influenza immunization.

Waldman RH, Bergmann KC, Stone J, Howard S, Chiodo V, Jacknowitz A, Waldman ER, Khakoo R.

In order to compare the antibody response in serum and secretions from healthy young subjects and the elderly (greater than 60 years), volunteers were immunized with the commercial inactivated influenza virus vaccine, by the usual (parenteral) route or orally. Also, young and old mice (mean age, 20 months) were orally immunized with live influenza virus. The older mice responded with a very slight rise in their serum and respiratory tract antibody levels compared with the young mice but showed no diminution in protection against lethal viral challenge. Elderly volunteers showed only slight serum antibody responses after parenteral immunization compared with the young. Neither group demonstrated a rise in serum antibody following oral immunization. With respect to the secretory IgA (SIgA) antibody response, certain differences were noted between the young and the elderly: the preimmunization levels of antibody to influenza virus were significantly greater in nasal secretions and saliva in the elderly as compared to the young volunteers, and the salivary antibody response was diminished in the elderly. This lack of a salivary antibody response in the elderly was explicable by the inverse relationship between the preimmunization SIgA antibody titers and the response to immunization. Oral immunization led to no more side effects than observed in the placebo control group.

9: Int J Immunopharmacol 1985;7(5):719-23

A synthetic vaccine against influenza with built-in adjuvanticity.

Shapira M, Jolivet M, Arnon R.

In a previous publication we demonstrated that an anti-viral response against influenza can be achieved by immunization with a conjugate of the synthetic peptide corresponding to the sequence 91-108 of the hemagglutinin, when administered in complete Freund's adjuvant. In the present study we compare the adjuvant activity of the synthetic MDP with that of CFA and alum, in the above mentioned immunological system. The level of anti-peptide antibodies raised by the three adjuvants was similar, with only slight variations, yet, only CFA led to significant cross reaction with the virus. Nevertheless, MDP, when linked covalently to the conjugate (91-108)-TT was an efficient substitute for CFA in inducing anti viral protection against in vivo challenge infection. The administration of free MDP in a mixture with the peptide-toxoid conjugate did not lead to a significant protection.

10: Int J Immunopharmacol 1983;5(5):403-10

Effects of mycobacterial fractions and muramyl dipeptide on the resistance of mice to aerogenic influenza virus infection.

Masihi KN, Brehmer W, Lange W, Ribi E.

The nonspecific protective effect in mice of pre-exposure to mycobacterial components and muramyl dipeptide three weeks before aerosol infection with influenza virus A/PR/8/34 (H1N1) was studied. Muramyl dipeptide, when combined with trehalose dimycolate and emulsified in an oil-in-water emulsion, conferred complete protection comparable to specific immunization with a high dose of formalin inactivated A/PR/8/34 influenza viral vaccine. Animals pre-exposed to muramyl dipeptide plus trehalose dimycolate showed a marked reduction in lung virus titres, an earlier clearance of detectable infectious virus, and an earlier onset of antibody production in comparison to control mice. Resistance to infection was also observed with BCG-cell wall skeleton combined with trehalose dimycolate and trehalose dimycolate alone when given as oil-in-water preparations. The route of administration of nonspecific stimulants was crucial. Only intravenous but not intradermal inoculation produced significant protection.

11: Clin Exp Immunol 1990 Dec;82(3):435-9

An experimental influenza subunit vaccine (iscom): induction of protective immunity to challenge infection in mice after intranasal or subcutaneous administration.

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An experimental influenza virus (A/PR/8/34(H1N1] vaccine was tested and evaluated in mice. The mice were inoculated once or twice intranasally or subcutaneously with 1 or 10 micrograms of iscoms prior to challenge with high dose of live virus. It was demonstrated that two intranasal administrations were as efficient as two s.c. administrations, both routes inducing high levels of antibody and protection against challenge infection. With a one-dose regimen, the s.c. route induced a somewhat higher antibody response than the intranasal route; this might be explained by technical difficulties connected with an intranasal administration.

12: Vaccine 1990 Dec;8(6):595-9

Cross-protection against influenza B type virus infection by intranasal inoculation of the HA vaccines combined with cholera toxin B subunit.

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The relationship between the antibody responses to various influenza B type virus HA vaccines and protection against live B virus infection was investigated in Balb/c mice which had been inoculated intranasally with a combination of the HA vaccines and B subunit of cholera toxin (CTB) 4 weeks previously. The inoculation of HA vaccine, prepared from B/Ibaraki/2/85 (B/Ibaraki), B/Nagasaki/1/87 (B/Nagasaki) or B/Aichi/5/88 (B/Aichi) viruses, combined with

CTB induced high levels of both nasal IgA and serum HI antibodies to any of B/Ibaraki, B/Nagasaki and B/Aichi viral antigens. Simultaneous inoculation of each CTB-combined HA vaccine provided complete protection against B/Ibaraki virus infection which is demonstrated by both rapid clearance of pulmonary virus and complete survival. On the other hand, the inoculation of HA vaccine prepared from B/Yamagata/16/88 (B/Yamagata) virus together with CTB induced only a low level of nasal IgA antibodies, cross-reactive to B/Ibaraki, B/Nagasaki and B/Aichi viral antigens and protected only partially against B/Ibaraki virus challenge. The involvement of the B type virus-specific immunity in this protection was suggested by the absence of protection against B/Ibaraki virus infection in mice previously inoculated with both A/PR/8/34 (H1N1) virus HA vaccine and CTB. These results suggest that antibodies to various influenza B viruses are cross-reactive to each B type virus antigens and that cross-protection against B virus infection could be conferred depending on the degree of B type virus cross-reactive immunity including secretory IgA antibodies.

13: Vaccine 1990 Aug;8(4):347-52

Enhancement of the protective efficacy of inactivated influenza A virus vaccine in aged mice by IL-2 liposomes.

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A dose-dependent, vaccine-induced protection of aged and young Balb/c mice against lethal influenza A virus challenge has been demonstrated. Low dose formalin-inactivated influenza A virus vaccine was protective in young mice, but not in aged mice, while a higher dose was protective in both. Administration of low dose vaccine with IL-2 liposomes conferred protection comparable to the high dose in aged mice. Serum neutralizing antibody responses were stimulated by vaccine in a dose-dependent manner while IL-2 liposomes significantly enhanced responses in the low dose paralleled protection in young but not in aged mice. Lung interferon levels paralleled lung virus titres in young but not in aged mice. CTL responses in infected mice were generally higher in young than aged mice. These results demonstrate efficacy of IL-2 liposomes as an adjuvant for influenza virus vaccines in the aged.

14: Vaccine 1990 Jun;8(3):243-8

Comparison of intranasal inoculation of influenza HA vaccine combined with cholera toxin B subunit with oral or parenteral vaccination.

Hirabayashi Y, Kurata H, Funato H, Nagamine T, Aizawa C, Tamura S, Shimada K, Kurata T.

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The antibody responses to influenza virus A/PR/8/34 HA vaccine and protection against virus

challenge in mice given the vaccine together with the B subunit of cholera toxin (CTB) intranasally were compared with those in mice given the vaccine with CTB perorally, intraperitoneally or subcutaneously. Intranasal vaccination induced remarkably higher levels of antiviral IgA antibodies in both respiratory washings and serum than did other routes of vaccination. The titres of antiviral IgG antibodies in respiratory washings and serum, and haemagglutination-inhibiting (HI) antibodies in serum, were similar after intranasal and parenteral vaccination. Oral vaccination, however, induced low levels of antiviral IgG antibodies but no detectable HI antibodies. Moreover, intranasal immunization elicited significantly higher titres of antiviral IgA antibodies in intestinal secretions in comparison with oral immunization. In contrast, parenteral immunization failed to induce these IgA antibodies. In virus challenge studies, a greater protective effect was seen after intranasal and intraperitoneal vaccination than after other routes of vaccination. These results suggest that intranasal inoculation of combined HA vaccine and CTB is superior to oral or parenteral inoculation in protecting mice. Furthermore, the intestinal antiviral IgA responses suggest that intranasal administration of CTB-combined vaccines could be effective not only against respiratory pathogens but also against enteropathogens.

15: Virus Res 1990 Apr;16(1):83-93

Comparison of inactivated, live and recombinant DNA vaccines against influenza virus in a mouse model.

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The protective efficacy of influenza hemagglutinin expressed from recombinant vaccinia virus was compared with that induced by inactivated or infectious influenza vaccines. Intraperitoneal and intranasal routes of vaccination were compared. All the vaccines except the intranasally administered, inactivated vaccine induced detectable levels of neutralizing and hemagglutination-inhibiting antibodies in the serum of mice at 28 days postvaccination. Immunization with any of the intranasally administered vaccines reduced the amount of influenza virus nucleoprotein antigen in lungs after challenge with a homologous, mouse-adapted strain of influenza virus. Intraperitoneally administered vaccines failed to provide such protection. These results indicated that the route of vaccine administration may be the most critical factor for inducing protective immunity. The results also showed that in this mouse model a recombinant DNA-based vaccine could provide protection equivalent to that provided by conventional attenuated and inactivated influenza vaccines.

16: Vaccine 1990 Apr;8(2):159-63

Effect of immunomodulator adamantylamide dipeptide on antibody response to influenza subunit vaccines and protection against aerosol influenza infection.

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Adamantylamide dipeptide (AdDP) is a novel synthetic compound combining the antiviral properties of amantadine and the essential adjuvant activity of immunomodulator muramyl dipeptide. Mice were immunized with influenza A/Taiwan/1/86 (H1N1), A/Sichuan/2/87 (H3N2) and influenza B/Beijing/1/87 subunit vaccines containing AdDP or aluminium hydroxide (Al(OH)3). Induction of homologous haemagglutination-inhibition (HI) antibodies and correlation to protection against lethal aerosol influenza A/PR/8/34 (H1N1) infection were investigated. Subunit vaccine containing A/Sichuan (H3N2) and Al(OH)3 stimulated high HI antibody titres but failed to provide protection against heterologous influenza A (H1N1) challenge infection following either the primary or the secondary immunizations. In contrast, similar treatment with A/Sichuan subunit vaccine containing AdDP conferred significant protection against heterologous challenge despite low levels of circulating antibody. Primary immunization with even influenza B/Beijing subunit vaccine containing AdDP, but not Al(OH)3, provided partial protection against influenza A challenge. These results suggest that appropriate immunomodulators like AdDP can convert restricted homotypic immunity induced by inactivated influenza subunit vaccines to advantageous cross-reacting type of heterologous response.

17: J Virol 1990 Mar;64(3):1370-4

Vaccination with inactivated influenza A virus during pregnancy protects neonatal mice against lethal challenge by influenza A viruses representing three subtypes.

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A single intraperitoneal injection of pregnant mice with a monovalent Formalin-inactivated influenza A virus vaccine protected their offspring against a lethal challenge dose of the same influenza A virus H3N2, H2N2, and H1N1 subtypes, as well as against challenge with the other two subtypes. Degree of protection was vaccine dose related. Cross-fostering of neonates indicated that protection was conferred by breast milk antibodies. Serum virus-specific neutralizing antibodies in the mothers and neonates correlated with resistance to vaccine virus, but were detected against other subtypes only in a complement enhancement test or when high doses of vaccine were given.

18: Vaccine 1993;11(10):987-93

Efficacy of equine influenza vaccines for protection against A/Equine/Jilin/89 (H3N8)--a new equine influenza virus.

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A new H3N8 equine influenza virus [A/Equine/Jilin/1/89 (Eq/Jilin)] appeared in Northeastern China in 1989 and caused high mortality in horses; the available evidence indicates that it has not yet spread outside this region of the world. Serological analysis with postinfection ferret sera in haemagglutination inhibition (HI) tests confirmed that Eq/Jilin is antigenically distinct from H3N8 equine influenza viruses isolated between 1963 and 1991 and also showed that a current equine influenza virus [A/Equine/Alaska/1/91 (H3N8)] had undergone antigenic drift. In the present study we determine if vaccine against a recent H3N8 influenza virus [A/Equine/Kentucky/1277/90 (Eq/Kentucky)] that was standardized for haemagglutinin content will protect mice against lethal challenge with the new H3N8 influenza virus from China. Complete protection is defined as prevention of virus replication in the lungs of mice 3 days after challenge. High doses of Eq/Kentucky vaccine in aqueous suspension (0.5-5.0 micrograms HA per dose) provided minimal protection against Eq/Jilin challenge as judged by virus titres in the lungs of vaccinated animals. Eq/Kentucky vaccine in adjuvant (1.0-5.0 micrograms HA per dose) did provide complete protection against challenge with Eq/Jilin in mice. Eq/Jilin vaccine in aqueous suspension induced complete protection of mice against challenge with Eq/Kentucky at doses from 0.5 to 5 micrograms HA and in adjuvant doses of Eq/Jilin from 0.1-5.0 micrograms HA were efficacious. Homologous protection against Eq/Jilin or Eq/Kentucky was induced by doses of vaccine from 0.5-5.0 micrograms HA per dose in aqueous suspension and from 0.01-5.0 micrograms HA per dose in adjuvant.(ABSTRACT TRUNCATED AT 250 WORDS)

19: J Immunol 1992 Aug 1;149(3):981-8

Cross-protection against influenza virus infection afforded by trivalent inactivated vaccines inoculated intranasally with cholera toxin B subunit.

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Cross-protection against influenza virus infection was examined in mice, immunized intranasally with a nasal site-restricted volume of inactivated vaccines together with cholera toxin B subunit (CTB) as an adjuvant. The mice were challenged with either a small or a large volume of mouse-adapted virus suspension, each of which gave virgin mice either a predominant upper or lower respiratory tract infection. A single dose of a monovalent influenza A H3N2 virus vaccine with CTB provided complete cross-protection against the small-volume challenge with a drift virus within the same subtype, but a slight cross-protection against the large-volume challenge. A second dose of another drift virus vaccine increased the efficacy of cross-protection against the large-volume challenge. Similar cross-protection against H1N1, H3N2, or B type drift virus challenge was provided in the mice having received a primary dose of a mixture of H1N1, H3N2, and B virus vaccines with CTB and a second dose of another trivalent vaccine. The degree of cross-protection against the small- and the large-volume infection paralleled mainly the amount of cross-reacting IgA antibodies to challenge virus hemagglutinin in the nasal wash and that of cross-reacting IgG antibodies in the bronchoalveolar wash, respectively. On the other hand, in mice immunized subcutaneously with the trivalent vaccines having no cross-reacting IgA antibodies, the efficacy of cross-protection was not so high as that of nasal vaccination. These results suggest that the nasal inoculation of trivalent vaccines with CTB provides cross-protection against a broader range of viruses than does the current parenteral vaccination.

20: Lab Anim Sci 1992 Jun;42(3):222-32

Influenza virus infections and immunity: a review of human and animal models.

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Studies of the pathogenesis of influenza infection have involved the extensive use of animal models. The development of the current concepts of immunity to influenza and of the contribution the secretory immune system makes toward the protection of mucosal surfaces against influenza infection would have been impossible without this use of animals. The pathology and clinical signs of influenza infection in both natural and experimental hosts, the advantages and disadvantages of the most common experimental influenza infection models, and the contribution of animal models to the understanding of local and systemic immunity to influenza infection are discussed.

21: J Virol 1992 Feb;66(2):1162-70

A novel particulate influenza vaccine induces long-term and broad-based immunity in mice after oral immunization.

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The immunogenicity of a novel particulate oral influenza vaccine was examined in terms of antibody response and protection in mice. Oral immunization with chicken erythrocytes (CRBC) adsorbed with gamma-irradiated influenza A virus induced high levels of immunoglobulin G antibodies and protection in the lung compared with gamma-irradiated virus administered alone or CRBC. Immunoglobulin A antibodies were the predominant antibodies in nasal washings, and their presence did not correlate with protection as well as immunoglobulin G antibodies. Immunity was not specific for the immunizing virus subtype, as antibodies and enhanced lung clearance of virus were demonstrated with different virus subtypes. However, mice were not protected when challenged with live influenza B virus. The antibody response and the degree of protection were dependent on both the concentration of virus adsorbed to CRBC and number of CRBC adsorbed to virus. Virus-adsorbed CRBC given subcutaneously failed to induce antibodies or protection. Oral immunization with A/Qld/6/72 (H3N2) virus gave a high level of protection over 12 weeks, which could be demonstrated with different subtypes. Protection correlated with antibody levels in the lung determined by both enzyme-linked immunosorbent and hemagglutination inhibition assays, although the levels detected by the latter declined over time.

22: Vaccine 1994 Nov;12(14):1340-8

Influenza A subtype cross-protection after immunization of outbred mice with a purified chimeric NS1/HA2 influenza virus protein.

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Influenza A/PR/8/34-derived chimeric (D) protein (SK&F 106160) composed of the first 81 amino acids (aa) of NS1 fused to the conserved 157 C-terminal aa of HA2 (NS1 1-81-HA2 65-222) was previously shown to induce H-2d-restricted protective cytotoxic T-lymphocyte (CTL) immunity in inbred mice. However, D protein, like other small peptides, exhibited haplotype dependence and was not immunogenic in H-2b and H-2K mice. A potential use of this antigen in humans and the role of T cells in any protection were evaluated in outbred Swiss and inbred CBF6F1 (H-2d/b) mice. Mice immunized with D protein and challenged by small-particle aerosol with a lethal dose of influenza virus were significantly protected against mortality from influenza A/H1N1 and A/H2N2 ($p < 0.05$-< 0.0000001), but not from A/H3N2 and influenza B viruses when compared with control mice. D protein did not induce serum virus-neutralizing antibody but caused virus to be cleared faster in immunized mice. Protection was long-lasting. In vivo depletion of either Lyt2 (CD8+) or L3T4 (CD4+) T cells with monoclonal antibodies led to abrogation of in vitro-generated CTL activity in CF6F1 mice and significant reduction in the protective efficacy of D protein against virus challenge in both Swiss and CF6F1 mice. These results suggest that protection was mediated by CD8+ and/or CD4+ cells and not antibody. Thus D protein, via a conserved sequence on the HA2 polypeptide, has the potential to induce partially cross-reactive CTL that may protect against influenza virus disease in humans.

23: Vaccine 1994 Sep;12(12):1083-9

Escherichia coli heat-labile enterotoxin B subunits supplemented with a trace amount of the holotoxin as an adjuvant for nasal influenza vaccine.

Tamura S, Asanuma H, Tomita T, Komase K, Kawahara K, Danbara H, Hattori N, Watanabe K, Suzuki Y, Nagamine T, et al.

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Escherichia coli heat-labile enterotoxin B subunit (LTB) (2 micrograms), supplemented with a trace amount of the holotoxin (LT) (0.02-20 ng), was examined for the adjuvant effect on antibody (Ab) responses against influenza inactivated haemagglutinin (HA) vaccine in Balb/c mice. Each mouse received a primary intranasal (i.n.) inoculation with the vaccine (1.5 micrograms), prepared from PR8 (H1N1) virus, together with LT-containing LTB and in 4 weeks a second i.n. inoculation of the vaccine alone. The inoculation of the vaccine with the LT-containing LTB induced significantly high primary and secondary anti-HA IgA and IgG Ab responses in the nasal wash and the serum, while the vaccine with LTB or less than 2 ng of LT induced little response. The synergistic adjuvant effect was maximal in the concentration of LTB supplemented with 0.2-2 ng of LT. Under these conditions, the augmented IgA and IgG Ab responses, which are cross-protective to PR8 HA molecules, provided complete cross-protection

against PR8 virus challenge in mice immunized with heterologous vaccine within the same subtype. These results suggest that LTB containing a trace amount of LT can be used as a potent adjuvant for nasal vaccination of humans against influenza.

24: Vaccine 1994 Jul;12(9):791-7

Selective induction of protection against influenza virus infection in mice by a lipid-peptide conjugate delivered in liposomes.

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We have previously reported (Muller et al. Vaccine 1990, 8, 308) that two cyclic peptide analogues called D loop and K loop, corresponding to residues 139-147 in site A of the haemagglutinin (HA) of influenza A virus (strain X31), were both able to provide protective immunity to infected OF1 mice when administered in the form of peptide-ovalbumin conjugates. The predicted conformation of the D loop is nearly identical to that of the native loop known from the X-ray structure of HA, while the predicted conformation of the K loop differs significantly from the native one. In this study, the two peptides were conjugated to small unilamellar liposomes, thus creating a chemically defined immunogen, and OF1 mice were immunized with these liposomes containing monophosphoryl lipid A as adjuvant. Compared with protein carrier systems, the liposomal preparations are completely synthetic and avoid the use of Freund's adjuvant. By using liposomes associated with the D loop, we were able to achieve 70% protection of the mice against intranasal challenge with the influenza virus while no protection was obtained with the liposome-associated K loop. The difference in effect between the two liposome and ovalbumin carrier systems may result from the induction of different structures in the peptides when coupled to lipid anchors than when coupled to proteins.

25: J Med Microbiol 1994 Apr;40(4):261-9

IgG subclass response and protection against challenge following immunisation of mice with various influenza A vaccines.

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The serum total IgG and IgG subclass and nasal wash IgA and IgG antibody responses of mice to influenza virus A/Hong Kong/68 (H3N2) subunit preparations administered parenterally as a single dose, incorporated either in immune stimulatory compounds (ISCOMs) or liposomes with Freund's Complete Adjuvant, or as an aqueous material, as well as to live, infectious virus were measured by ELISA at 10 days and 3, 5, 7 and 22 weeks after immunisation. The protection of the upper and lower respiratory tracts provided by these preparations against homologous and heterologous challenge infection was assessed. Of the four variously-presented subunit

preparations, influenza subunit ISCOMs induced relatively high and persisting levels of each of the different IgG subclasses, particularly IgG2a, throughout the study, and most nearly approached those observed after intranasal infection of mice with infectious virus. Furthermore, nasal wash IgA and IgG antibody levels, particularly at 5 or 7 weeks after immunisation, were also significantly greater in mice given the subunit ISCOM preparation than those induced by other subunit preparations with adjuvant or subunits given alone, and provided protection of both the upper and lower respiratory tracts against challenge as similar to that elicited by infectious virus.

26: Vaccine 1994 Mar;12(4):310-6

Formulation of inactivated influenza vaccines for providing effective cross-protection by intranasal vaccination in mice.

Tamura S, Asanuma H, Ito Y, Yoshizawa K, Nagamine T, Aizawa C, Kurata T.

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Attempts were made to formulate an inactivated influenza vaccine to provide effective cross-protection by intranasal vaccination in mice. Mice were immunized with a nasal site-restricted volume of various HA vaccines (split-product virus vaccines), prepared from some of the H1N1 subtype viruses which circulated in humans from 1934 to 1986, together with cholera toxin B subunit (CTB) as an adjuvant. Four weeks later, they were challenged intranasally with a lethal dose of the earliest H1N1 virus strain, A/PR/8/34 (PR8) or the latest virus strain, A/Yamagata/120/86 (Yamagata/86). The adjuvant-combined vaccines, prepared from drift H1N1 viruses, A/Kumamoto/37/79 and A/Bangkok/10/83, provided a higher degree of cross-protection against a challenge with Yamagata/86 than with PR8. A booster with another drift virus vaccine given 4 weeks after the primary vaccination increased the protection against Yamagata/86; the effect was higher when mice were vaccinated with a later strain as the second antigen than when boosted with PR8. These results suggest that vaccination with a later virus strain followed by another later strain in a two-dose nasal vaccination regimen gives effective cross-protection against the current epidemic virus strains.

27: Acta Virol 1997 Oct;41(5):251-7

Cross-protection of mice immunized with different influenza A (H2) strains and challenged with viruses of the same HA subtype.

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Cross-protection of mice immunized with inactivated preparations of human and avian influenza A (H2) viruses was determined after lethal infection with mouse-adapted (MA) variants of human A/Jap x Bell/57 (H2N1) and avian A/NJers/78 (H2N3) viruses. The MA variants differed from the original strains by acquired virulence for mice and changes in the HA antigenicity.

These studies indicated that mice vaccinated with human influenza A (H2) viruses were satisfactorily protected against challenge with A/Jap x Bell/57-MA variant; the survival rate was in the range of 61%-88.9%. Immunization of mice with the same viral preparations provided lower levels of protection against challenge with A/NJers/78-MA variant. Vaccination of mice with the avian influenza A (H2) viruses induced better protection than with human strains against challenge with both MA variants. Challenge with A/NJers/78-MA variant revealed that 76.2%-95.2% of animals were protected when vaccinated with avian influenza virus strains isolated before 1980, and that the protection reached only 52.4%-60.0% in animals vaccinated with strains isolated in 1980-1985. The present study revealed that cross-protection experiments in a mouse model could provide necessary information for the development of appropriate influenza A (H2) virus vaccines with a potential for these viruses to reappear in a human population.

28: Vaccine 1995 Oct;13(14):1353-9

Intranasal immunization of mice against influenza with synthetic peptides anchored to proteosomes.

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Synthetic vaccines that are based on peptides representing immunogenic epitopes require a carrier molecule as well as an adjuvant in order to be effective. The choice of carriers or adjuvants approved for use in humans is very limited, and a considerable effort is devoted to develop new and efficient delivery systems. One of these vehicles utilizes preparations of outer membranes of meningococci, that form hydrophobic interactions, denoted proteosomes. Immunogenic proteins and peptides can be anchored to these proteosomes vesicles, which may serve as both carrier and adjuvant functions. In the present study we examined the ability of proteosomes to present epitopes of influenza, to elicit specific anti-influenza responses and to protect mice against viral challenge after intranasal immunization. Three influenza peptides were used--one corresponding to amino acid residues 91-108 of the haemagglutinin surface glycoprotein of H3 subtype, which comprises a B-cell epitope, and two from the internal nucleoprotein--a T-helper cell (Th) epitope (residues 55-69) and a cytotoxic T-lymphocyte (CTL) epitope (147-158). Mice were immunized intranasally (i.n.) with preparations containing each of the above epitopes, or various combinations thereof. The results obtained with this system demonstrate that influenza epitopes represented by synthetic peptides anchored to a proteosome carrier elicit both humoral and cellular specific immune responses, that can lead to partial protection of the mice from viral challenge. The importance of immunizing with vaccines containing both B- and T-cell peptide epitopes was emphasized by the demonstration that such vaccines elicited longer lasting immunity and led to more effective protection against influenza viral challenge.

29: Vaccine 1995 Jul;13(10):927-32

Protection of mice against lethal viral infection by synthetic peptides corresponding to B- and T-cell recognition sites of influenza A hemagglutinin.

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Previously, we reported 12 synthetic T- and B-cell recognition regions representing surface areas of the hemagglutinin (HA) of X31 influenza virus. In the present study, four of these peptides were examined in Balb/c mice for their ability to produce protective immunity against lethal infection with a dose equivalent to 10 LD50 of influenza virus. These peptides corresponded to the following sequences: 23-36 (HA1-1); 138-152 (HA1-3); 183-199 (HA1-6) and 1-11 (HA2-10). Each of the selected peptides, in their free form, evoked anti-peptide antibodies that cross-react with intact X31 virus. Two of the peptides, HA1-1 and HA1-3, also elicited virus-specific delayed type hypersensitivity (DTH) responses. These two peptides, when injected into mice, not only failed to protect the immunized mice against challenge with influenza virus, but in fact caused greater susceptibility to viral infection as compared to control animals that had been injected with saline. In contrast, peptides HA1-6 and HA2-10, which were unable to induce adequate virus-specific DTH responses, conferred 42-46% and 54-73% protection, respectively, compared to the control group that received only saline ($P < 0.03$ to $P < 0.01$).

30: Infect Immun 1995 Mar;63(3):961-8

Synthetic peptides representing T-cell epitopes act as carriers in pneumococcal polysaccharide conjugate vaccines.

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Improvement of antibody responses to polysaccharides through their linkage to proteins is thought to be mediated by protein-specific T helper (Th) cells. To investigate whether the carrier protein of a conjugate could be substituted by a Th epitope, Streptococcus pneumoniae type 17F polysaccharide (PS) was bromoacetylated and coupled to different peptides via their carboxy-terminal cysteines. Two peptides, one from the mycobacterial 65-kDa heat shock protein (hsp65) and the other from influenza virus hemagglutinin, are well-known Th epitopes. Two other peptides were selected from the pneumolysin sequence by Th epitope prediction methods; one of them was synthesized with cysteine either at the carboxy or the amino terminus. Three conjugates consistently elicited in mice anti-PS immunoglobulin M (IgM) and IgG responses that were not observed upon immunization with derivatized PS without peptide. The same conjugates induced no anti-PS antibody responses in athymic (nu/nu) mice, whereas clear responses were elicited in euthymic (nu/+) controls, demonstrating the thymus-dependent character of these conjugates. Only the three conjugates inducing anti-PS responses were capable of eliciting antipeptide antibodies. One of the immunogenic conjugates was studied in more detail. It induced significant protection and an anti-PS IgG response comprising all subclasses. On the basis of these results and proliferation studies with peptide and conjugate-primed cells, it is concluded that linkage of Th epitopes to PS in the right orientation enhances its immunogenicity in a thymus-dependent manner. Future possibilities for using peptides as carriers for inducing

antibody responses to poorly immunogenic saccharide antigens are discussed.

31: Vaccine 1995 Feb;13(3):253-60

Immune response of mice to immunization with subunit influenza A vaccine in DTP vaccine.

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Experiments were carried out to examine the initial feasibility of immunizing infants and children with inactivated influenza virus vaccine combined with diphtheria-tetanus-pertussis (DTP) vaccine. Groups of mice were immunized with saline vaccine or vaccine mixed with DTP: three doses of vaccine were given 3 weeks apart, and the antibody response and resistance to challenge infection were tested 3 weeks after the 1st and 3rd immunizations. The results showed that the antibody response and immunity to challenge virus infection were significantly greater for mice given vaccine in DTP than for mice given saline vaccine alone. Comparison of the response to graded doses of vaccine in saline or DTP indicated that vaccine in DTP was > 250-fold more effective in inducing serum antibody and protection than saline vaccine alone. The enhancing activity of DTP was significant for the alum component alone; however, most of the adjuvant effect was from the antigen components of the DTP vaccine. The results suggest that immunization against influenza in infants and young children could be achieved by combining small amounts of influenza antigen with DTP vaccines; however, the present results have been obtained in mice, and, since the responses to vaccines and adjuvants vary from species to species, the present results cannot be used to indicate similar results in human volunteers. The results indicate the potential value of an immunization procedure which should be tested in volunteers and which could provide a simple strategy for the immunization of at-risk infants and children against influenza.

32: Vaccine 1995 Feb;13(2):155-62

Mucosal immunoadjuvant activity of liposomes: induction of systemic IgG and secretory IgA responses in mice by intranasal immunization with an influenza subunit vaccine and coadministered liposomes.

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This paper reports on a novel immunoadjuvant activity of liposomes. An influenza subunit preparation, containing the isolated viral surface antigens, was incorporated in a liposomal formulation. Administration of this vaccine to mice via the intranasal (i.n.) route resulted in a stimulated serum IgG response relative to the response to i.n. immunization with the antigen alone. In addition, the liposomal vaccine induced a secretory IgA (sIgA) response in the mucosa

of the lungs and nasal cavity. Both serum IgG and sIgA responses persisted up to at least 21 weeks postimmunization. Immune stimulation was observed with negatively charged liposomes consisting of phosphatidylcholine (PC), cholesterol and dicetylphosphate (DCP), but not with zwitterionic liposomes, consisting of PC and cholesterol alone. Remarkably, for stimulation of serum IgG responses and induction of an sIgA response, liposomes could be simply mixed with the antigen. Moreover, i.n. administration of empty liposomes up to 48 h prior to i.n. immunization with the subunit antigen also resulted in immune stimulation, indicating that the liposomes did not exert their adjuvant effect by acting as a carrier for the antigen. The liposomal vaccine conferred protection against infection. It is concluded that liposomes, administered i.n., provide a promising adjuvant system for stimulation of antibody responses in general, and mucosal sIgA responses in particular.

33: Vaccine 1995;13(15):1399-402

Protection of mice against influenza A virus challenge by vaccination with baculovirus-expressed M2 protein.

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We have investigated the potential of the conserved transmembrane M2 protein of influenza A/Ann Arbor/6/60 virus, expressed by a baculovirus recombinant, to induce protective immunity in BALB/c mice. Vaccination of mice with M2 shortened the duration of virus shedding and protected mice from a lethal infection with A/Ann Arbor/6/60 virus but not B/Ann Arbor/1/55 virus, suggesting that the protection was mediated by an M2-specific mechanism. Serum antibodies were detected which reacted with synthetic peptides defining three antigenic determinants located on both the external N- and internal C-termini of the M2 protein. Furthermore, vaccination with M2 protected mice from death following a lethal challenge with the heterologous A/Hong Kong/68 (H3N2) virus. These results demonstrate the potential to elicit heterosubtypic immunity to type A influenza viruses through vaccination with a conserved transmembrane protein.

34: Vaccine 1995 Jan;13(1):3-5

Cross-protection against influenza virus infection in mice vaccinated by combined nasal/subcutaneous administration.

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The effects of a combination of intranasal (i.n.) and subcutaneous (s.c.) administration of inactivated influenza vaccine for priming and boosting on the cross-protection against antigenically drifted virus challenge were examined in Balb/c mice. Mice were primed through

the i.n. or s.c. route with a CTB*-A/Kumamoto/37/79 (H1N1) combined vaccine (CTB*: cholera toxin B subunit supplemented with 0.2% of the holotoxin) and boosted through the i.n. or s.c. route with another drift virus vaccine, A/Bangkok/10/83 (H1N1), 4 weeks later. Two weeks after boosting, the mice were challenged with a third drift virus, A/Yamagata/120/86 (H1N1). The combination of i.n. priming and i.n. boosting afforded the highest cross-protection, while combinations of s.c. priming and i.n. or s.c. boosting afforded little cross-protection. In parallel with the protective activity, anti-A/Yamagata haemagglutinin-reactive IgA and IgG antibodies were detected in nasal and bronchoalveolar wash specimens. These results suggest that cross-protection against a variant virus challenge is most favourably provided by i.n. priming with the CTB* combined vaccine and i.n. boosting with the vaccine, which optimally induces cross-protective IgA and IgG antibodies.

35: J Med Microbiol 1995 Jan;42(1):53-61

Comparative antibody responses and protection in mice immunised by oral or parenteral routes with influenza virus subunit antigens in aqueous form or incorporated into ISCOMs.

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The total and subclass antibody responses of mice and protection of these animals against live influenza A/Sichuan/2/87 virus challenge infection were determined after immunisation with homologous A/Sichuan/87 aqueous or ISCOM-formulated surface glycoprotein subunit antigens administered by either the oral or intramuscular routes. The results show that the greatest systemic and local antibody responses were elicited in mice immunised with A/Sichuan ISCOMs by the intramuscular route; protection against homologous virus challenge was also effective in these animals, particularly after two doses of the vaccine. However, relatively high immune responses and protection were also elicited by the A/Sichuan/87 ISCOM vaccine administered orally. Immunisation of mice by the intramuscular route resulted in levels of serum IgG2a subclass antibody significantly greater than those induced by the same preparation given by the oral route, or by the aqueous A/Sichuan/87 subunit antigen preparation administered by either route. The findings indicate that the ISCOM delivery system can be used for immunisation by the oral route, although in mice, under the conditions used, this strategy compares unfavourably with the intramuscular route in terms of both local and systemic immune responses and protection against homologous challenge virus infection.

36: Arch Virol 1995;140(6):1015-31

Immunogenicity of influenza and HSV-1 mixed antigen ISCOMs in mice.

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Immunostimulating complexes (ISCOMs) were prepared with mixtures of antigens from influenza A virus (A/PR/8/34 or A/Sichuan/2/87) and herpes simplex virus type 1 (HSV-1), and were characterised by enzyme linked immunosorbent assay (ELISA) and electron microscopy using double-labelling immunogold techniques employing monoclonal antibodies to influenza or HSV-1 glycoproteins. The immunogenicity of the mixed antigen ISCOMs was evaluated in mice, following administration by the subcutaneous route, by measuring the total and subclass IgG antibody responses. Protection of these animals against challenge with live influenza A/Sichuan virus or live HSV-1, was compared with that induced by immunization with aqueous mixed antigen preparations. It was found that relatively high humoral responses to both influenza and HSV antigens, and increased levels of protection to both influenza and HSV viruses were elicited in mice receiving the mixed antigen ISCOM preparation compared to those observed in animals receiving the mixed aqueous subunit preparation. The findings also indicate that antigens from more than one virus can be used in an ISCOM formulation to produce immunity and protection.

37: Vaccine 1994 Dec;12(16):1541-4

Protective immunity by intramuscular injection of low doses of influenza virus DNA vaccines.

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Dose-response relationships were investigated between dose of influenza virus haemagglutinin (HA) or nucleoprotein (NP) DNA vaccines, and immunogenicity and protective efficacy based on humoral and cellular immunity. In mice, intramuscular (i.m.) injection of HA or NP DNA, at doses of 100 ng to 1 microgram, was found to generate haemagglutination inhibiting (HI) antibodies and cytotoxic T-lymphocytes, respectively, and provide protection in influenza virus challenge models. A direct correlation between the amount of DNA injected and the level of HI antibody was observed. In non-human primates, high-titre neutralizing antibodies were induced in animals vaccinated with as little as 10 micrograms of HA DNA. These results indicate that low doses of DNA administered by i.m. injection provide protective efficacy against influenza.

38: Proc Natl Acad Sci U S A 1994 Nov 8;91(23):11187-91

Enteric immunization of mice against influenza with recombinant vaccinia.

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Intrajejunal administration to mice of a recombinant vaccinia virus containing the influenza virus hemagglutinin gene induced IgA antibody in nasal, gut, and vaginal secretions. It also induced IgG antibody in serum and cell-mediated immunity. The immunization provided significant protection against an influenza virus challenge. This work suggests that enteric-coated

recombinant vaccinia could be an orally administered, inexpensive, multivalent, temperature-stable, safe, and effective vaccine for children that could be particularly useful in developing nations, where multiple injections are not easily administered. Oral administration of vaccines should also reduce children's fear of shots at the doctor's office.

39: J Virol 1997 May;71(5):3391-6

Cross-protection among lethal H5N2 influenza viruses induced by DNA vaccine to the hemagglutinin.

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Inoculation of mice with hemagglutinin (HA)-expressing DNA affords reliable protection against lethal influenza virus infection, while in chickens the same strategy has yielded variable results. Here we show that gene gun delivery of DNA encoding an H5 HA protein confers complete immune protection to chickens challenged with lethal H5 viruses. In tests of the influence of promoter selection on vaccine efficacy, close correlations were obtained between immune responses and the dose of DNA administered, whether a cytomegalovirus (CMV) immediate-early promoter or a chicken beta-actin promoter was used. Perhaps most important, the HA-DNA vaccine conferred 95% cross-protection against challenge with lethal antigenic variants that differed from the primary antigen by 11 to 13% (HA1 amino acid sequence homology). Overall, the high levels of protection seen with gene gun delivery of HA-DNA were as good as, if not better than, those achieved with a conventional whole-virus vaccine, with fewer instances of morbidity and death. The absence of detectable antibody titers after primary immunization, together with the rapid appearance of high titers immediately after challenge, implicates efficient B-cell priming as the principal mechanism of DNA-mediated immune protection. Our results suggest that the efficacy of HA-DNA influenza virus vaccine in mice extends to chickens and probably to other avian species as well. Indeed, the H5 preparation we describe offers an attractive means to protect the domestic poultry industry in the United States from lethal H5N2 viruses, which continue to circulate in Mexico.

40: Vaccine 1997 Apr;15(5):541-6

The effect of Syntex adjuvant formulation (SAF-m) on humoral immunity to the influenza virus in the mouse.

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Syntex adjuvant in its microfluidized form (SAF-m) was equal to or superior to Freund's complete adjuvant in stimulating an enhanced hemagglutination inhibition (HI) antibody response in mice to trivalent influenza virus vaccine (TIV). There was an average 16-fold

increase in HI titer for the three components of the vaccine with no significant differences among strains. The increased serum antibodies correlated with an increase in protection against infection. The threonyl-MDP (t-MDP) component of the adjuvant played no role in this activity. The vehicle, in contrast, was so effective that it could be diluted 1:202 (in the presence of (t-MDP) and still retain a statistically significant effect. Vaccine and adjuvant could be stored together at 4 degrees C for 2 years without a statistically significant change in potency. Mice were given a priming immunization with TIV, PBS, or adjuvanted TIV (AIV). A year later, the mice were boosted with heterotypic TIV or AIV. The nature of the priming immunization made no difference in the strong antibody response to an AIV boost. However, priming significantly improved the response to TIV with AIV being the best primer. The enhancement in the antibody response to AlShanghai of the unprimed (PBS) elderly mice caused by AIV (14-fold improvement over TIV) was similar to that in young mice. Female mice had antibody titers which overall were 2.6-fold higher than those of males ($P < 0.0001$) for AIV and TIV.

41: J Virol 1997 Apr;71(4):2772-8

Genetically engineered live attenuated influenza A virus vaccine candidates.

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We have generated new influenza A virus live attenuated vaccine candidates by site-directed mutagenesis and reverse genetics. By mutating specific amino acids in the PB2 polymerase subunit, two temperature-sensitive (ts) attenuated viruses were obtained. Both candidates have 38 degrees C shutoff temperatures in MDCK cells, are attenuated in the respiratory tracts of mice and ferrets, and have very low reactogenicity in ferrets. Infection of mice or ferrets with either mutant conferred significant protection from challenge with the homologous wild-type virus. Three tests for genetic stability were used to assess the propensity for reversion to virulence: 14 days of replication in nude mice, growth at 37 degrees C in tissue culture, and serial passage in ferrets. One candidate, which contains mutations intended to reduce the ability of PB2 to bind to cap structures, was stable in all three assays, whereas the second candidate, which contains mutations found only in other ts strains of influenza virus, lost its ts phenotype in the last two assays. This approach has therefore enabled the creation of live attenuated influenza A virus vaccine candidates suitable for human testing.

42: J Infect Dis 1997 Feb;175(2):352-63

Adjuvant activity of the heat-labile enterotoxin from enterotoxigenic Escherichia coli for oral administration of inactivated influenza virus vaccine.

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Alternative strategies for vaccination against influenza that elicit both systemic antibody and

mucosal IgA responses are needed to improve the efficacy in protection against infection. This study demonstrated that oral delivery of inactivated influenza vaccine with the heat-labile enterotoxin (LT) from enterotoxigenic Escherichia coli elicited the spectrum of humoral and cell-mediated responses in BALB/c mice critical for the protection and recovery from influenza virus infection. Coadministration of LT with oral influenza vaccine increased antiviral serum IgG and mucosal IgA responses compared with administration of oral influenza vaccine alone. Serum hemagglutination-inhibition and neutralizing antibodies were also augmented by LT. The adjuvant potentiated protection from infection with influenza A H3N2 viruses in mouse lower and upper respiratory tracts, enabling the use of lower doses of oral vaccine. Coadministration of LT with oral inactivated influenza vaccine induced influenza virus-specific proliferative T cells, interleukin-2 production, and major histocompatibility complex class I-restricted cytotoxic T cells.

43: Vaccine 1997 Jan;15(1):71-8

Characterization of humoral immune responses induced by an influenza hemagglutinin DNA vaccine.

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We have examined in detail the characteristics of the humoral immune response and protective efficacy induced by an influenza hemagglutinin (HA) DNA vaccine. In mice injected intramuscularly with HA DNA, the magnitude of the immune responses generated, as measured by ELISA and hemagglutination inhibiting (HI) antibodies, was directly related to the amount of DNA injected and the number of doses administered. The level of anti-HA antibodies in DNA-vaccinated mice was higher than that in convalescent immune mice and was maintained for at least 1.5 years. The immunoglobulin isotype profile of the antibodies was predominantly IgG2a, similar to that induced by live virus infection but in contrast to the relative abundance of IgG1 antibodies observed after inoculation with formalin-inactivated whole virus. The presence of pre-challenge HI antibodies was found to be a good correlate of protection, in that every animal with a detectable HI titer was protected from a lethal challenge. Complete protection from a lethal dose of influenza virus (A/PR/34), as judged by 100% survival and no weight loss, was conferred by as little as 1 microgram of DNA (given twice). Furthermore, mice injected with 10 to 100 micrograms doses, when subsequently challenged with virus, showed no increase in HI titer and no production of antibodies directed against the challenge virus, suggesting a substantial inhibition of virus replication after challenge.

44: Virology 1996 Oct 1;224(1):10-7

Long-term maintenance of B cell immunity to influenza virus hemagglutinin in mice following DNA-based immunization.

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This study demonstrates that gene-gun inoculation of mice with DNA encoding the influenza virus hemagglutinin (HA) results in the life-long maintenance of protective B cell responses. Using a sensitive single-cell enzyme-linked immunospot assay, we show that all of the HA-specific plasma cells are localized in the bone marrow and spleen 1 year postimmunization. As a consequence of prior virus challenge, only a small population of antibody-forming cells was found in the lymphoid tissues associated with the respiratory tract. The tissue distribution of HA-specific plasma cells in these mice was identical to the profile in infected controls. Complete protection against live virus challenge in the aged vaccinated mice did not require prior exposure to virus. Thus, immunization with the DNA, vaccine provides long-term protective immunity against otherwise lethal infection.

45: J Immunol 1996 Oct 1;157(7):3039-45

Protection against lethal viral infection by vaccination with nonimmunodominant peptides.

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CTL response of H-2b mice to influenza PR8 virus is directed against the nucleoprotein (NP)-derived immunodominant 366-374 (NP366PR8) peptide presented by the Db molecule. However, NP has three nonimmunodominant peptides corresponding to the 17-25 (NP17), 55-63 (NP55), and 97-105 (NP97) sequences that have the Db consensus motifs and bind to the Db molecule with an intermediate (NP55) or low (NP17 and NP97) affinity. In a previous report, we have shown that NP55 peptide is naturally processed by infected cells. In the present work, we studied whether nonimmunodominant peptides can protect mice against viral infection. Antiviral protection was evaluated by measuring three parameters: survival after inoculation of a lethal dose of mouse-adapted PR8 virus, percentage of pulmonary lesions in surviving mice, and virus clearance from lungs of infected mice. Our results showed that immunization of B6 mice with nonimmunodominant peptides protected from PR8 virus infection, although less efficiently than immunization with the immunodominant NP366PR8 peptide. Protection was mediated by CD8 T cells. The efficacy of nonimmunodominant peptides correlated with their Db binding affinity; the low affinity binders NP17 and NP97 induced a weaker protection than the intermediate affinity binder NP55. A mixture of NP366PR8 and nonimmunodominant peptides gave a higher protection than NP366PR8 peptide alone. In conclusion, nonimmunodominant peptides protect against a viral infection with an efficacy that is proportional to their affinity for the restricting class I molecule.

46: Vaccine 1996 Apr;14(6):561-9

Recombinant neuraminidase vaccine protects against lethal influenza.

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The N2 neuraminidase gene of A/Victoria/3/75 influenza virus was engineered to encode a secretable protein (NAs) by replacing the natural N-terminal membrane anchor sequence with the cleavable signal sequence of the corresponding influenza hemagglutinin gene. Soluble NAs was expressed by a baculovirus/insect cell system and accumulated in the medium at levels between 6 and 8 microgram ml-1. A combination of biochemical and standard chromatographic techniques allowed the purification of NAs to homogeneity. Cross-linking analysis indicated that NAs was partly recovered as an authentic tetrameric protein, while the remaining fraction was composed of dimeric molecules and small amounts of monomeric NAs. Purified NAs was supplemented with low-reactogenic adjuvants and used to immunize mice. After a challenge infection with a lethal dose of homologous mouse-adapted X47 influenza virus, vaccinated animals showed resistance against severe disease symptoms and were protected from lethality. Based on the results of a passive immunization experiment, it may be concluded that performed antibody plays a central role in the mechanism by which vaccination with NAs confers viral protection.

47: Vaccine 1996 Jan;14(1):85-92

Synthetic recombinant influenza vaccine induces efficient long-term immunity and cross-strain protection.

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Synthetic vaccines utilize specific antigenic epitopes in order to elicit a protective immune response. In this work we examined the immunogenicity of chimeric proteins expressing influenza epitopes and their ability, as single products or in various combinations, to protect mice from viral challenge. Oligonucleotides coding for three epitopes (HA91-108, NP55-69 and NP147-158) stimulating B cells, T helper cells and cytotoxic T lymphocytes (CTLs), respectively, were individually inserted into the flagellin gene of a Salmonella vaccine strain. Immunization of mice with the resultant hybrid flagella resulted in a specific humoral or cellular response. The protective efficacy of the chimeric flagella was evaluated by intranasal immunization of mice, without any adjuvant, and subsequent challenge with infectious virus. The construct containing the B-cell epitope by itself led to partial protection. However, the addition of the two T-cell epitopes augmented the protection in a significant manner. The protective immunity conferred by this combined vaccine, comprising the three epitopes, persisted for at least 7 months after the last boost, and was effective against several influenza A strains. Furthermore, this vaccine fully protected mice from a lethal challenge, and enhanced their recovery process. Our results indicate that stimulation of the different arms of the immune system is required for effective anti-influenza response, and demonstrate the applicability of such synthetic recombinant approach for preparing a broad spectrum influenza vaccine.

48: Int Arch Allergy Immunol 1995 Dec;108(4):321-6

Synthetic recombinant vaccines against viral agents.

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Synthetic recombinant vaccines are expression vectors incorporating defined epitope(s) of microbial agents. They are prepared by inserting synthetic oligonucleotide(s) coding for previously identified relevant epitopes into the genome of a desired vector, using recombinant DNA technology. The results discussed indicate that immunization with such vaccines carrying viral epitopes may lead to protective immunity against viral agents. Oligonucleotides coding for three influenza epitopes stimulating B cells, T helper cells and cytotoxic lymphocytes were individually inserted into the flagellin gene of a Salmonella vaccine strain. Immunization of mice with the resultant recombinant bacteria or their isolated flagella induced a specific mucosal anti-influenza protective response. The most efficient vaccine consisted of all three recombinant flagella, administered intranasally. The protection elicited was cross-strain specific, long-lasting and efficient against a lethal viral challenge.